FORMULATION AND *IN VITRO* EVALUATION OF ZIDOVUDINE MICROSPHERES

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Peelamedu, Coimbatore – 641004

Certificate

This is to certify that the dissertation entitled FORMULATION AND *IN VITRO* EVALUATION OF ZIDOVUDINE MICROSPHERES was carried out by PRASATH.R, in the Department of Pharmaceutics, PSG College of Pharmacy, PSG Institute of Medical Sciences & Research, Peelamedu, Coimbatore, which is affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai, under the supervision and guidance of Prof. A. K. Chandrasekharan, M.Pharm., Principal, PSG College of Pharmacy, PSG IMS & R, Coimbatore.

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INTRODUCTION AND OBJECTIVE OF THE INVESTIGATION

Novel drug delivery systems are being employed both experimentally and therapeutically to alter the distribution of drugs in the body with an idea of delivering them more efficiently and reducing the toxicity of the existing drugs. One such area in which research is gaining much attention is “controlled drug delivery” because of its enhanced efficacy along with reduced toxicity.

It have been reported that 40% of the compounds developed in pharmaceutical industry are poorly soluble in water. A limiting factor to the *in vivo* performance of poorly water soluble drugs after oral administration is their inadequate ability to become wetter and getting dissolved in the gastrointestinal (GI) fluid.

Microspheres have more advantages over other soluble carrier’s liposomes. Liposomes have low drug entrapment efficiency, rapid leakage of water soluble drugs especially in the presence of blood components, poor stability and method of preparation which are not compatible with large scale production requirements. However microspheres can entrap various molecules in a stable and reproducible way.

Zidovudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Zidovudine is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The bioavailability of zidovudine can be increased by making the drug to get released in a controlled manner by incorporating the drug in certain biodegradable polymers.

The aim of this study was to formulate and evaluate microencapsulated controlled release preparations of Zidovudine using ethyl cellulose as the retardant material with high entrapment efficiency and extended release. Ethylcellulose is a natural, biodegradable polymer and the method adopted for preparing microsphere was water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion technique.

The plan of work was designed as follows:-

- Preparation of zidovudine microspheres using water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion technique.
- Determination of size and shape of microspheres.
- Estimation of percentage yield of the microspheres and is drug entrapment efficiency.
- Study of *in vitro* release from prepared microspheres.

INTRODUCTION

Over the years we have learnt the optimization of drug therapy is of very great importance and in the process patience convenience, compliance, safety has to be taken into account.

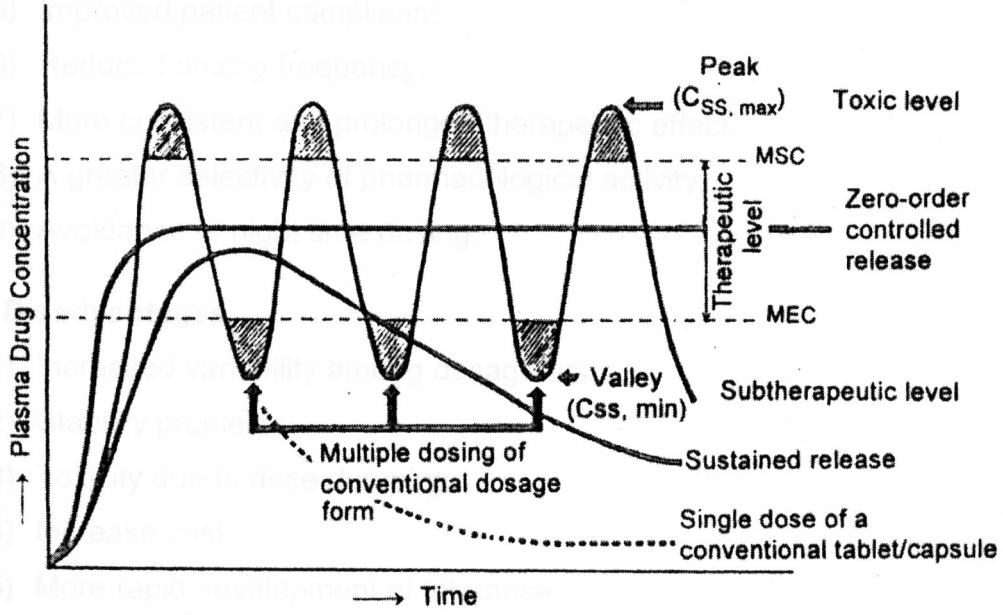
For many decades treatment of an acute disease or chronic illness has been mostly accomplished by deliver of drugs to patients using various pharmaceutical dosage forms like capsules, pills etc. These types of dosage forms require frequent dosing and thus causing inconvenience to patients.

There are three types of drug delivery i.e. controlled delivery, targeted delivery and modulated release. Targeted delivery refers to the systemic administration of drug carrier with the goal of delivering drug to specific cell types, tissues and organs. Controlled release refers to the use of delivery device with the objective of releasing the drug into the patient body at a predominant rate at a specific time or with specific release profiles. Modulated release implies release of the drug at variable rate which is controlled by various factors such as bio feedback, environmental conditions, sensor input or an external control device.

Controlled drug delivery is the one which delivers the drug at a specific rate locally or systemically for a specific period of time.

Controlled release dosage forms are being referred by a number of terminologies such as delayed action, extended action, prolonged release, slow release, gradual release, sustained release and timed release dosage forms.

Fig 1. Hypothetical plasma drug concentration-time profile



A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations

OBJECTIVE OF CONTROLLED RELEASE

- Increase in patient compliance by obtaining extended duration of action
- Targeted drug action by using carriers or chemical derivatization to deliver drugs to a particular target cell type.
- Localized drug action by spatial or controlled release system adjacent to or in the diseased tissue or organ.

Advantages

1) Decrease in toxicity and adverse effects.
2) More consistent and prolonged therapeutic effect.
3) Improved patience compliance.
4) Reduced dosing frequency.
5) More uniform blood concentration.
6) A greater selectivity in pharmacological activity.
7) Avoidance of night time dosing.
8) Better drug utilization.

Disadvantages

1) Stability problems.
2) Toxicity due to dose dumping.

3) Increased cost.

4) More rapid development of drug tolerance.

5) Increased variability among dosage units.

6) Poor *in vitro and in vivo* correlation.

7) Need for additional patient education and counseling.

8) Decreased systemic availability.

9) Retrieval of drug is difficult in case of toxicity or hypersensitivity reactions.

FACTORS GOVERNING THE DESIGN OF CONTROL RELEASE DOSAGE FORM:-

Drug related

- Aqueous solubility
- Partition coefficient
- Molecular size
- Drug stability
- Protein binding

Biological factors

- Absorption.
- Cardiac rhythm.

- Distribution.
- Elimination.
- Duration of action.
- Margin of safety.
- Side effects.
- Diseased sates.

Physiological factors

- Prolonged drug absorption.
- Variability in GI emptying time.
- GI blood flow.

Pharmacological factors

- Sensitivity/tolerance.
- Changes in drug effect upon multiple dosing.

Pharmacokinetic factors

- Dose dumping.
- First pass metabolism.
- Enzyme induction/inhibition upon multiple dosing.
- Variability in urinary pH and its effect on drug elimination. (Jain., 1997)

CLASSIFICATION OF ORAL CONTROLLED RELEASE SYSTEMS:

A) Continuous release systems

1) Dissolution controlled systems

   a) Matrix type

   b) Reservoir type

2) Diffusion controlled release systems

   a) Matrix type

   b) Reservoir type

3) Dissolution and diffusion controlled release systems.

4) Ion exchange resin drug complexes.

5) Osmotic pressure controlled systems.

6) Slow dissolving salts and complexes.

7) pH dependant formulations

8) Hydrodynamic pressure controlled systems.

B) Delayed transit and continuous release systems.

1) Altered density systems

   a) High density.

   b) Low density.

   c) Floating.

2) Mucoadhesive systems.

3) Size based systems.

C) Delayed release systems.

1) Intestinal release systems.

2) Colonic release systems.

Future developments in controlled drug delivery

The most exciting opportunities in controlled drug delivery lies in the area of responsive delivery systems, with which it will be possible to deliver drugs through implantable devices in response to a measured drug level or to deliver a drug precisely to a targeted site. Much of the developments of novel materials in controlled delivery are focusing on the preparation and use of the responsive polymers with specifically designed microscopic and macroscopic structural and chemical features.

Such systems include,

- Copolymers with desirable hydrophilic/hydrophobic interactions
- Complexation networks responding via hydrogen or ionic bonding
- Block or graft copolymers.
- Dendrimers or star polymers as nanoparticles for immobilization of enzymes, drugs, peptides, or other biological agents.

MICROENCAPSULATION

Microencapsulation is one of the most intriguing fields in the area of drug delivery systems. It is an interdisciplinary field that requires knowledge of the field of pure polymer science, familiarity with emulsion technology and an in-depth understanding of drug and protein stabilization. This method is also used to make controlled release dosage forms. Solids, liquids, gels can be entrapped inside one or more polymeric coatings. The structure of microcapsules depends on the type of manufacturing process and mostly spherical. Pellets, liposomes, multiple emulsions, microcapsules and microspheres are common examples of microcapsulate drug delivery systems. (Edith mathiowitz., 2002)

Table: 1 Classification of microencapsulation methods

PROCESS	COATING MATERIAL	SUSPENDED MEDIUM
Interfacial polymerization	Water soluble & insoluble monomers	Aqueous/organic solvent
Complex coacervation	Water soluble polyelectrolyte	Water
Coacervation	Hydrophobic polymers	Organic solvent
Thermal denaturation	Proteins	Organic
Salting-out	Water soluble polymer	water
Solvent evaporation	Hydrophilic or hydrophobic polymers	Organic solvent or water
Hot melt	Hydrophilic or hydrophobic polymers	Aqueous/organic solvent
Solvent removal	Hydrophilic or hydrophobic polymers	Organic solvents
Spray drying	Hydrophilic or hydrophobic polymers	Air, Nitrogen
Phase separation	Hydrophilic or hydrophobic polymers	Aqueous/organic solvent.

(i) Reasons for microencapsulation

- ❖ Sustained and controlled release
- ❖ Taste and odor masking
- ❖ Protection of drug against environment
- ❖ Delay of volatilization
- ❖ Enteric coating

- ❖ Detoxification
- ❖ Conversion of oils and other liquids into solids for ease of handling
- ❖ Separation of flow of powders
- ❖ Isolation from tissues
- ❖ Safe handling of toxic substances

(ii) Properties of drugs used in microencapsulation

The core of microcapsules formulated may contain one or more drugs either a single drug or combination of drugs with suitable additives to form a liquid or solid phase. Liquid core may be composed of polar or non polar substantial that comprises the active ingredient or that acts as vehicle for dissolved or suspended drugs. The solvent properties of liquids critically influence the rate of drug release and selection of coating materials. For drugs of low solubility with known bioavailability problems associated with low rates of dissolution, decrease in particle size of suspended drugs may be important in enhancing *in vivo* absorption. Smaller microparticles also have faster release rates because of their increased surface area per unit volume or weight of core material. Solid cores are used more frequently than liquid cores. Very small core particles tend to give tend to give aggregation problem during production, because of the attraction force present at the surface of the

particles. Larger particles can cause problems because of their rapid sedimentation rate. The shapes of these cores are also important. Density of cores is very important for controlling the transit time in GI tract. Increasing the density was the most important factor in prompting the retention of pellets in stomach. Decreasing density is important in floating type dosage forms. Swelling of the core with disruption of coating leads to uncontrolled drug release.

(iii) Properties of polymers used for microencapsulation

The selection of appropriate coating material dictates to a major degree the resultant physical and chemical properties of the microcapsules. The coating material should be capable of forming a film that is cohesive with the core material and the ideal coating material should be compatible and non reactive with the core material. The polymer coating should provide desired coating properties such as strength, impermeability, optical properties, flexibility and stability. (Jain., 1997).

MICROSPHERES AND MICROCAPSULES

Microspheres can be defined as spherical empty particles ranging in size from 1 to 1000 micrometer. Microspheres are characteristically free flowing powder consisting of proteins and synthetic polymers, which are biodegradable in nature.

Microcapsules consist of a well defined core and a well defined envelope; the core can be solid, liquid or gas, the envelope is made of continuous process or non porous polymeric phase.

It is also defined as a spherical particle with size varying from 50 nm to 2 mm containing a core substance.

Microspheres have been investigated for intravenous and intra arterial targeting and delivery systems. Microspheres and Microcapsules have been injected in the vessels to ensure passive targeting of the drugs. The drug release is controlled by diffusion through the polymer matrix and/or by erosion of the polymer. The role of microspheres and microparticles depend on their size and site of injection.

Microparticles of diameter smaller than 2μm can be injected in an intravenous, intra arterial and intra peritoneal manner in order to target the reticuloendothelial system (RES). Intravenous injection of Microspheres of

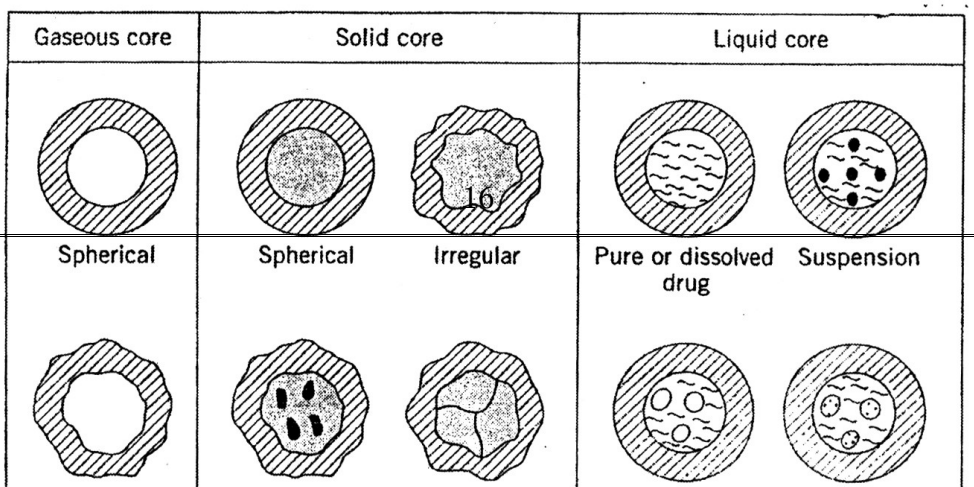
size from 3-12 micrometer is intended to block the capillaries of the lungs, liver and spleen.(Vyas., 2002)

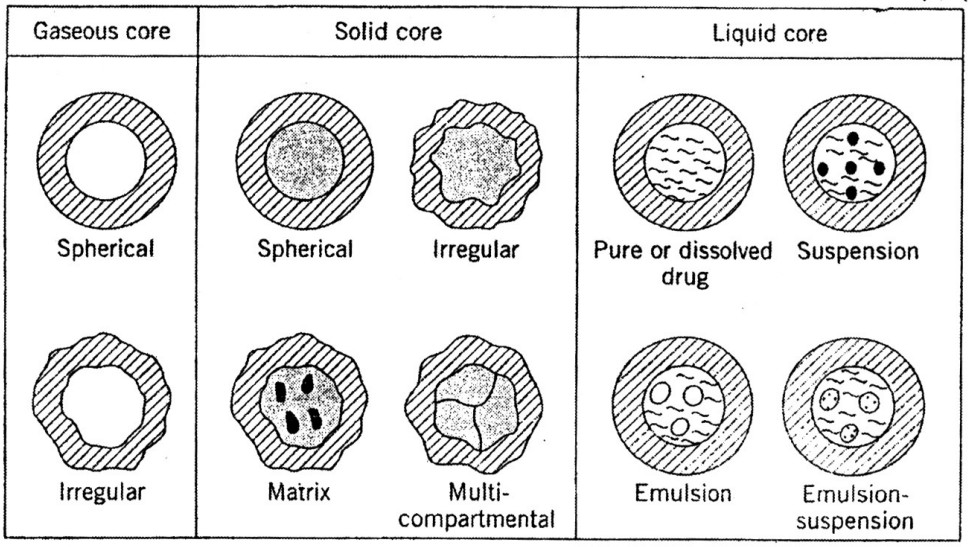
Vessels can be hyperselectively embolized with drug loaded particulate materials of more than 10 micrometer. Microspheres of 100-300 micrometer size are the most appropriate embolic agents. They reach the intra lesional precapillary arteries and cause reduction of blood flow.

Drug incorporation methods

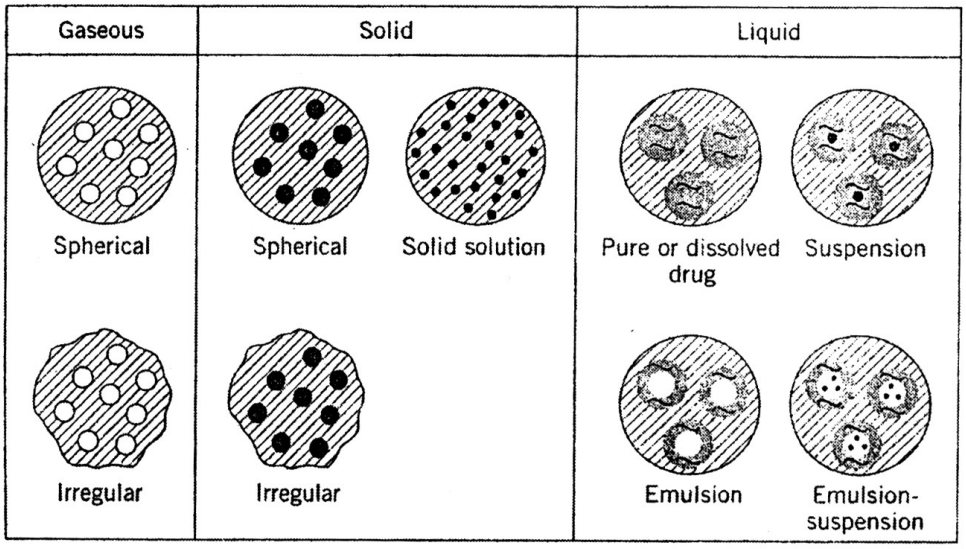
1. The drug can be dissolved or homogeneously dispersed in the polymer (Matrix device-microsphere type).
2. Drugs can be dissolved or homogenously dispersed in the polymer (Matrix device- Microsphere type).

Fig: 2 various configurations of a)microcapsules and b)microspheres




Gaseous core
Solid core
Liquid core
Spherical
Spherical
Irregular
Pure or dissolved drug
Suspension
Irregular
Matrix
Multi-compartmental
Emulsion
Emulsion-suspension

(a)


Gaseous
Solid
Liquid
Spherical
Spherical
Solid solution
Pure or dissolved drug
Suspension
Irregular
Irregular
Emulsion
Emulsion-suspension

(i) Advantages of microspheres

1. Taste and odor masking.
2. Protection of the drugs against the environment (moisture, light, heat, and/or oxidation) and vice versa (prevention of pain on injection).
3. Delay of volatilization.
4. Conversion of oils and other liquids to solids for ease of handling.
5. Separation of incompatible materials (other drugs or excipients such as buffers)
6. Safe handling of toxic substances.
7. Improvement of flow of powders.
8. Aid in dispersion of water-insoluble substances in aqueous media.
9. Production of sustained release, controlled release and targeted medications.

(ii) Route of administration

Route of administration is selected depending on the drug properties, disease states being treated and the age condition of the patient. Desirable properties of microspheres to be used for delivery will also change depending on route of administration.

a) Oral delivery

Oral delivery is the simplest route of drug administration. Constraints of the oral route are numerous on the whole it offers potential danger than parental route. The relatively brief transit time of about 12 hours through the GI tract limits the duration of action that can be expected via the oral route. Recently, it has been reported that microspheres of less than 10μm in size are taken up by peyer's patches and may increase the retention time in the stomach. Also microspheres made of polymers with mucoadhesive properties get attached to stomach or intestine and prolong the residence time in the stomach. Bioavailability of drugs with limited solubility in the stomach or intestine and small absorption rate constant can be increased by increasing the retention time in the stomach. Improved drug delivery was observed in mucoadhesive propertied microspheres when compared to non mucoadhesive propertied microspheres administered alone.(Jain., 1997)

b) Parenteral delivery

Most of the microsphere based controlled delivery systems are developed with the aim of using them for parenteral administration. Drug released is completely absorbed in this case. Microspheres used for parenteral delivery should be sterile and should be dispersible and compatible in a suitable vehicle for injection. Surfactants in small concentration are often

necessary for reconstituting hydrophobic particles for injection in aqueous vehicles, which are reported for certain adverse tissue reactions and affect the release of incorporated drug.

PROCESS

Polymers used

Synthetic polymers

Non-biodegradable

- PMMA
- Acrolein
- Glycidyl methaacrylate
- Epoxy polymers

Biodegradable

- Lactides and glycolides
- Polyalkyl cyano acrylates
- Polyanhydrides

NATURAL MATERIALS

Proteins

- Albumin
- Gelatin
- Collagen

Carbohydrates

- Starch
- Agarose
- Chitosan

Chemically modified carbohydrates

- DEAE cellulose
- Poly (acryl dextran)
- Poly(acryl starch)

Prerequisites for ideal microparticulate carriers

- Longer duration of action
- Control of content release
- Increase of therapeutic efficacy
- Protection of drug

- Reduction of toxicity
- Biocompatibility
- Sterilizability
- Relative stability
- Dispersability or water solubility
- Bioresorbability
- Targetability
- Polyvalent

(iv) Preparation of microspheres

- The preparation of microspheres should satisfy certain criteria,
- The ability to incorporate reasonably high concentrations of drug.
- Stability of the penetration after synthesis with clinically acceptable shelf life.
- Controllable particle size and dispersability in aqueous vehicles for injection.
- Release of active ingredient with good control over a wide time scale.
- Biocompatibility with a controllable biodegradability.
- Susceptibility to chemical modification.

(v) Methods of preparation

The method of preparation and its choice are equivocally determined by some formulation and technology related factors as mentioned below,

- The particle size requirement.
- The drug or protein should not be adversely affected by the process.
- Reproducibility of the release profile and the method.
- No stability problem.
- There should be no toxic products associated with the final product.

1. Solvent evaporation

This technique is based on the evaporation of the initial phase of an emulsion by agitation. Initially the polymeric supporting material was dissolved in a volatile organic solvent. The active ingredient that has to be encapsulated is then dispersed of and dissolved in organic solution to form suspension, emulsion or solution. In the following step the organic phase is emulsified under agitation in a dispersing phase consisting of the non solvent of the polymer, which is immiscible with the organic solvent, which contains an appropriate tension active additive. Once emulsion is stabilized, agitation is maintained and solvent is the creation of solid microspheres. On the completion of solvent evaporation process, the microspheres held in

suspension in the continuous phase recovered by filtration of centrifugation and are washed and dried.

2. Thermal and cross linking

Microspheres made from natural polymers are prepared by a cross linking process; the polymers include gelatin, albumin, starch and dextran. Water in oil emulsion is prepared, where the water phase is a solution of the polymer, which contains the drug to be incorporated. The oil phase is a suitable vegetable oil or oil-organic solvent mixture containing an oil- soluble emulsifier. Once the desired water-oil emulsion is formed, the water soluble polymer is solidified by some kind of cross linking process. This requires a thermal treatment or the addition of chemical cross linking agent like glutaraldehyde. In the chemical and thermal cross linking process both the amount of chemical and intensity of heat applied are critical in determining the swelling properties and release profiles of microspheres. If glutaraldehyde is used as cross linking agent, residual amounts can have toxic effects.

3. Precipitation

A variation on the evaporation method is the precipitation method. The emulsion consists of polar droplets dispersed in a non polar medium. Solvent may be removed from the droplets by the use of a co solvent. The resulting increase in the polymer-drug concentration causes a precipitation forming a suspension.

4. Freeze drying

The technique involves the freezing of the emulsion; the relative freezing points of the continuous-phase solvent is usually organic and is removed by sublimation at low temperature and pressure. Finally, the dispersed phase solvent of the droplets is removed by sublimation, leaving polymer-drug particles.

3. Spray drying and spray congealing

Spray drying and spray congealing methods are based on the drying of the mist of polymer and drug in air. Depending upon the removal of the solvent or the cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in an organic volatile solvent such as dichloromethane, acetone etc. the drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The

atomization leads to formation of small droplets or the fine mist form which the solvent evaporates instantaneously leading to the formation of microspheres in a size range of 1-100μm. Microspheres are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying.(Jain., 1997).

Very rapid solvent evaporation, however leads to the formation of microparticles.

Advantages

→ Feasibility of the operation under aseptic conditions.

→ The process is rapid.

→ Suitable for both batch and bulk manufacturing.

→ This technique can be use to encapsulate large number of drugs.

4. Phase separation and coacervation techniques

Phase separation method is specially designed for preparing the reservoir type of the system, i.e. to encapsulate water soluble drugs e.g. peptides, proteins, however some of the preparations are of matrix type particularly, when the drug is hydrophilic in nature e.g. steroids. In matrix type device, the drug or the protein is soluble in polymer phase. The process is based on the principle of decreasing the solubility of the polymer in the

organic phase to affect the formation of the polymer rich phase called coacervates. The coacervation can be brought about by addition of the third component to the system which results in the formation of two phases, one is rich in polymer and the other, i.e. supernatant, depleted of the polymer. This is achieved by the addition of the salt, non-solvent addition, addition of the incompatible polymer or change in pH.

In this technique, the polymer is first dissolved in a suitable solvent and then the drug is dissolved by making its aqueous solution. Phase separation is then accomplished by changing the solution conditions by using any of the method above. The process is carried out under continuous stirring to control the size of microparticles.

(V) Loading of drug

The active components are loaded over the microspheres principally using two methods, i.e. during preparation of the microsphere or after the formation of microspheres by incubating them with drug or protein. The active component can be loaded by means of the physical entrapment, chemical linkage and surface absorption.

Maximum absorption can be achieved by incorporating the drug during the time of preparation but it may get affected by many other processes such as method of preparation, presence of additives (e.g. cross

linking agent, surfactants, stabilizers etc), the loading is carried out in preformed microspheres by incubating them with a very high concentration of drug in a suitable solvent. The drug gets loaded in these microspheres through diffusion or penetration through the pores in the microspheres as well as adsorption in their surface. The solvent is then removed leaving the drug loaded microsphere.

The Freundlich model is applied to determine the adsorption of the drugs. The Freundlich equation is,

$$X/M = KC^{p}_{eq}$$

Where,

K= Constant related to the capacity of adsorbent for the adsorbate.

P= Constant related to the affinity of the adsorbent for the adsorbate.

(vi) Release of drug from microspheres

The rate of drug release from the microspheres dictates their therapeutic action. Drug release is governed by the molecular structure of the drug and the resistance of polymer to degradation and the surface area and porosity of microspheres.

Reservoir delivery systems extend the residence time of drug within the systemic circulation originally focused on zero-order dissolution kinetics.

In porous polymeric systems the rate of drug release is dictated by the device surface area, which is linked directly to its shape. The rate of release from microspheres may be result of polymer erosion or diffusion.

The internal structure may vary as a function of microencapsulation process employed. Reservoir microcapsules have a core of drug coated with polymer. The drug is distributed homogenously throughout the polymeric matrix in monolithic microspheres.

The mechanism of drug release at a controlled rate from microspheres include diffusion of drug through a polymeric excipients, diffusion of trapped drug as polymer erodes, and release of drug through pores in the polymeric excipients. Increasing the surface area, by reducing the particle size, results in an increased release rate. The path length travelled by the drug in the matrix can be controlled by manipulating the microsphere loading. Microspheres with high drug content release the outline ingredient more rapidly than those with a low load. Thicknesses of the polymer also influence release rate.

Mechanism of drug release

Factors affecting the release of drug from the particulate system in relation with drug, microspheres and bio environment.

Drug

- Position in microspheres.
- Molecular weight.
- Concentration.
- Interaction with matrix.

Microspheres

Type and amount of matrix polymer.

- Size and density of microspheres.
- Extent of cross linking, denaturation or polymerization.
- Adjuvants.

Environment

- pH.
- Polarity.
- Presence of enzyme.

The geometry of carrier, i.e., whether it is reservoir type where the drug is present as a core, or matrix type in which drug is dispersed throughout the carrier, governs overall release profile of drug or active ingredients. (Jain., 1997).

SYSTEMS

Reservoir type system

Release from the reservoir type system with rate controlling membrane proceeds by first penetration of water through the membrane followed by dissolution of the drug in the penetrating dissolution fluid. The dissolved drug after partitioning through the membrane diffuses across the stagnant diffusion layer. The release is essentially governed by the Fick's first law of diffusion as,

$$J = -D\frac{dc}{dx}$$

Where,

J = flux per unit area.

D = diffusion coefficient.

Diffusion across the membrane determines the effectiveness of the carrier system. The release rate from the carriers can be modified by changing both the composition and thickness of the polymeric membrane.

Matrix type system

Release profile of the drug from this type of the device critically depends on the state of drug whether it is dissolved or dispersed in the polymer matrix.

In case of the drug dissolved in the polymeric matrix, amount of drug, and the nature of polymer affect the release profile. If this is the case the amount of drug appearing in the receptor phase at "t" is approximated by two separate equations. The first equation determines the 60% of drug release profile at the later stage.

$$\frac{dMt}{dt} = 2\,Mx\,(D/\pi \int^2 t)1/2$$

$$\frac{dMt}{dt} = \text{ 8 DM x / } \int^2 \exp\pi^2 D_t + / \int^2$$

Where,

l = thickness of polymer slab.

D = diffusion coefficient.

Mx = total amount of drug present in the matrix.

Mt = amount of drug released in time "t".

When the drug is dispersed throughout the polymer matrix then the release profile follows higuchi's equation.

$$\frac{dM_t}{dt} = \frac{A}{2}\,(2DC_sC_o)$$

Where,

A = area of matrix.

Cs = solubility of the drug in matrix.

Co = total concentration in the matrix.

(iv) Fate of microsphere in the body

The knowledge about the fate of microspheres after parenteral administration is very important for designing a drug delivery system. The biological fate of administered microspheres has been studied by radiolabelling techniques. ^{14}C, ^{131}I, ^{125}I, and ^{99}Tc are being used for radiolabelling. Fluoroscein labeling has also been found useful for studying the fate of microspheres *in vivo*.

Table: 2 proposed mechanism for uptake of microspheres

SITE	SIZE RANGE	FATE
Enterocyte/Endocyte	<220 nm	RES uptake
Paracellular uptake	100-200 nm	Unknown
Intestinal macrophage	1 µm	MLN
Persorption	5-150 µm	Blood and Excretory fluids
Peyer's Patches	20 nm - 10 µm	PP and MLN
Follicle associated epithelium	< 750 nm	MLN

(v) Applications of microspheres.

- Microspheres in vaccine delivery
- Antigen release
- Immune system
- Targeting using particulate carriers (ocular, intranasal, oral)
- Magnetic microspheres, Imaging
- Monoclonal antibodies.
- Microsponges: topical porous microspheres.(Jain., 1997).

ETHYL CELLULOSE MICROSPHERES

Ethyl cellulose microspheres were prepared based on the principle of double emulsion solvent diffusion technique with certain modifications. The rational for selecting EC for the shell material was that, this substance is commonly used as an additive in foods and drug because of its high inertness, and forms a stable, semi-permeable capsular membrane.

Ethyl cellulose is synthetic polymer; synthetic polymers have the advantage that they can be easily and reproducibly prepared. This can be copolymerized with one another to alter the physical, chemical and mechanical properties and can be prepared as low or high molecular weight material by suitable reaction conditions. Chemical bonds which are susceptible to degradation include amides, esters, orthoesters, acetyls,

glycosides and related groups. biodegradability of the polymer depends on many factors such as polymer structure, molecular weight ,physical form of the polymer and environment in which the polymer is placed since many proteolytic enzymes specifically catalyzes the hydrolyses of peptide linkages adjacent to substituted proteins, substituted polymers containing benzyl, hydroxyl ,carboxyl-methyl and phenyl groups have been prepared to improve biodegradability.

DOUBLE EMULSION SOLVENT DIFFUSION TECHNIQUE:

The technique was based on formation of an emulsion by agitation. Initially the drug and the polymeric material are dissolved in the solvent. Then the primary emulsion was formed by adding water little by little into the solvent, drug and polymer mixture which stirring was done simultaneously using a mechanical stirrer. After the emulsion was formed the stirring was stopped. Then the oily phase was taken along with surfactant and the primary emulsion formed was added to the oil phase slowly while stirring was continued at a constant speed. The process was continued for two hours and then a stabilizing agent was added in order to harden the preformed microspheres and stirring was further continued for one more hour.

Then the product formed was washed with an suitable compound and then filtered in order to remove the oily phase and air dried.

REVIEW OF LITERATURE

Chowdary *et al.*, (1989) prepared on microcapsules using calcium alginate and reported that method based on emulsification of a solution of sodium alginate containing the drug in an immiscible liquid medium followed by curing with calcium chloride to result in spherical calcium alginate microcapsules was reported. Aspirin, diazepam and nitrofurantoin were encapsulated by this method. The microcapsules were found to be slow and spread over extended period of time. The release mechanism was found to be of diffusion type.

Khawla abu-izza *et al.*, (1996) prepared zidovudine-loaded (AZT-loaded) sustained release microspheres and optimized using response surface methodology. Entrapment efficiency, yield, and percentage of loose surface crystals were investigated. All the investigated response variables were found to be highly dependent on the formulation variables, with strong interactions observed between the formulation variables. It was found that optimum overall desirability of AZT microspheres can be obtained at low levels of SDS and ethyl acetate concentrations and at intermediate levels of drug to polymer ratio. An optimized formulation was prepared under these

experimental conditions and evaluated for individual responses and overall desirability. It is clear that the loading efficiency was

highly dependent on the D: P ratio. As the theoretical loading increased, the loading efficiency increased significantly. A fixed amount of AZT is probably lost to the aqueous phase during the formation of microspheres and this loss obviously has a more detrimental effect on the loading efficiency of microspheres with relatively lower drug content.

Perumal., (2001) proposed a method to prepare modified release of ibuprofen by emulsion solvent diffusion technique. The technique was optimized for following process variables: the absence or presence of baffles in the reaction vessel, agitation rate and drying time. Thereafter, the influence of various formulation factors on the microencapsulation efficiency, *in vitro* drug release and micrometric properties were examined. The variables included the methaacrylic polymer, Eudragit RS 100, ibuprofen and the volume of ethanol used during microencapsulation. The results obtained were then interpreted on a triangular phase diagram to map the region of microencapsulation, as well as those formulations that yielded suitable modified release ibuprofen microspheres.

Gonzalez-Rodriguez *et al.*, (2002) prepared Alginate /chitosan particulate systems for diclofenac sodium release by ionic gelation (Ca^{2+} and Al^{3+}) and characterized by scanning electron microscopy and differential scanning calorimetry. The release of diclofenac sodium was prevented at acidic pH, while it was complete in a few minutes when pH is raised up to 6.4 and 7.2.The alginate / chitosan ratio and nature of gelifying cation allow a control of the release rate of the drug.

Bhupender Singh *et al.*, (2002) Designed, developed and optimized controlled release microcapsules of diltiazem hydrochloride reported that, microcapsules were formulated as per factorial design taking rate controlling coat polymer and emulgent that is ethylcellulose and span 80 respectively. The release of drug follows fickian drug release. The release was found to be quite regulated for controlled release purpose ($t_{80\%} \approx 9.5$hr) with little dose dumping (release up to 16hr$\approx$ 99%).

Sajeev *et al.*, (2002) formulated and evaluated microencapsulated preparations of diclofenac sodium (DFS) using different proportions of ethyl cellulose (EC) as the retardant material to extend the release. The formulated microcapsules were then compressed into tablets to obtain controlled release oral formulation. Phase separation coacervation technique was used to

prepare microcapsules of DFS using different proportions of EC in cyclohexane Physical characteristics of microcapsules and their tablets, *in vitro* release pattern of the designed microcapsules and their tablets prepared from them were studied using USP dissolution apparatus (USP 2000) type II (paddle method) in triple distilled water. The prepared microcapsules were white free and owing and spherical in shape. All tablets were good of quality with respect to appearance, drug content uniformity, hardness, weight variation, friability, and thickness uniformity a good correlation was obtained between drug release (t_{60}) and proportion of in the microcapsules. In the case of tabletted microcapsules, very good correlation could be established between release rate constant (k) and proportion of EC. All the formulations were highly stable and possessed reproducible release kinetics across the batches.

Mishara *et al*., (2003) prepared chitosan – alginate microcapsules for colon specific delivery of metronidazole and reported that microcapsules prepared by calcium chloride cross – linking method with different concentration of sodium alginate and chitosan. Then they were treated for three different coating with reduced molecular weight chitosan, guar gum and enteric coatings with cellulose acetate pthalate. Chitosan concentration significantly affected the strength and flexibility of membrane. Drug loading

was decreased with increase in the weight of either encapsulating polymer or chitosan and different coatings. *In vitro* drug release was found to be decreased with increasing chitosan and sodium alginate concentrations. Among the three coatings reduced molecular weight chitosan coating gave much lower drug release and exhibit colon specificity.

Sharbaraya *et al*., (2003) prepared chitosan microspheres of metaprolol tartarate by phase separation emulsification technique and microspheres are investigated for sustained release. Percentage yield was found to be 80-86 percent. The size range of microspheres varied from 3.5-31.5 µm. the *in vitro* release studies were carried out at different pH for a period of 10 hours and was compared with that of pure drug.

Dandagi *et al.,* (2004) prepared microcapsules of verapamil hydrochloride by Ionotropic gelation technique and reported that, increase in speed of rotation of calcium chloride (counter-ion) solution, leads to decrease in pellet size. Also it was found that with the increase in harvesting time, the pellet formed in turn decreased the drug entrapment efficiency. The release of the drug from micro pellets was found to be following non-Fickian diffusion mechanism, which accounts for the prolonged release of Verapamil Hydrochloride.

Chowdary *et al.*, (2004) prepared ethyl cellulose microspheres of glipizide by industrially feasible emulsion-solvent evaporation technique and the microspheres were investigated. The microspheres were discrete and free flowing. Encapsulation efficiency was in the range of 81-91 percent. Glipizide release from microspheres was slow, diffusion controlled and extended over a period of 10 d depending on the core: coat ratio, wall thickness and size of microspheres. In the *in vivo*, the microspheres produced a sustained hypoglycemic effect 6 d in normal rabbits. These microspheres were found to be suitable for parenteral controlled release.

Cui-Yun Yu *et al.*, (2005) prepared alginate based microparticles for the sustained release of antineoplastic drugs. The drug loaded microparticles were fabricated using a very convenient method under very mild conditions, i.e., directly shredding the drug loaded beads into microparticles in a commercial food processor. The mean sizes of the obtained microparticles were between 100 and 200μm. To effectively sustain the drug release, alginate microparticles were reinforced by chitosan during gelation. The drug release from the chitosan-reinforced alginate microparticles was obviously slower than that from the unreinforced microparticles. The effect of the reinforcement conditions on the drug release property of the microparticles was studied, and the optimized concentration of chitosan solution for

reinforcement was identified. The effects of drug feeding concentration and pH value of the release medium on the drug release were investigated. The *in vitro* release shows that chitosan reinforcement could effectively sustain the release of the drugs with low molecular weights. And the efficiency of reinforcement is affected by the concentration of chitosan solution.

Martinac *et al.*, (2005) prepared loratidine-loaded microspheres by spray drying of dispersions, emulsions and suspensions differing in polymeric

composition and solvents used. Composed microspheres were obtained by spray drying of two phase systems of Chitosan and ethyl cellulose (EC). Microspheres differed in EC/CM weight ratio (0:1, 1:2 and 1:3) and in loratidine/polymers weight ratio (1:6 and 1:8). Tensile studies showed that both, EC/CM ratio, and the type of spray dried system influenced the bioadhesive properties of microspheres in a way that microspheres with higher Chitosan content were more bioadhesive and microspheres prepared from suspensions were more bioadhesive than those prepared from emulsions, regardless of same polymeric combination.

Gohel *et al.*, (2005) Prepared and optimized sugar crosslinked gelatin microspheres of Diclofenac sodium reported that sugar (e.g. glucose, fructose) can induce cross linking of gelation for the preparation of modified release microspheres. The microspheres which were prepared by emulsion

crosslinking method revealed that, the parameters such as drug to gelation ratio, volume of light liquid paraffin and stirring rate were found to affect the morphology and drug release of microspheres.

Sunit Kumar Sahoo *et al.*, (2005) prepared Eudragit microspheres of stavudine by solvent evaporation technique. The prepared microspheres were characterized for their micromeritic properties and drug loading, as well by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry, x-ray powder diffractometry and scanning electron microscopy. The drug-loaded microspheres showed 67-91% of entrapment and release was extended up to 6 to 8 h. The infrared spectra and differential scanning calorimetry thermographs showed stable character of stavudine in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. The best-fit release kinetics was achieved with Higuchi plot followed by zero order and First order. The release of stavudine was influenced by the drug to polymer ratio and particle size & was found to be diffusion controlled.

Malay Kumar Das *et al.*, (2006) prepared zidovudine-loaded ethylcellulose microspheres by w/o/o double emulsion solvent diffusion method with high entrapment capacity and sustained release is described. The prepared microspheres were characterized by entrapment efficiency, *in vitro* release behavior, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). The drug-loaded microspheres showed 32 and

55% entrapment capacity. The DSC thermograms confirmed the absence of any drug-polymer interaction. SEM studies showed that the microspheres were spherical and porous in nature. The *in vitro* release profiles from microspheres of different polymer-drug ratios were best fitted to Higuchi model with high correlation coefficient and the n value obtained from Korsmeyer-Peppas model was ranged between 0.23 and 0.54. The drug release was found to be diffusion controlled mechanism.

Sameer Sharma *et al.*, (2006) prepared low density multiparticulate system for pulsatile release of meloxicam and reported that floating pulsatile drug delivery system was developed using porous calcium silicate (Florite RE) and sodium alginate, for time and site specific drug release of meloxicam. Drug adsorbed Florite RE powder was used to prepare calcium alginate beads by ionotropic gelation method, using 3 factorial designs and evaluated. The floating time was controlled by density of beads and hydrophobic character of drug. A pulsatile release of meloxicam was demonstrated by a simple drug delivery system which could be useful in chronopharmacotherapy of rheumatoid arthritis.

Das *et al.*, (2006) prepared Zidovudine-ethylcellulose microspheres were prepared by water-in-oil-in-oil double emulsion solvent diffusion method. Spherical free flowing microspheres having an entrapment efficiency of 32-54% were obtained. The effect of polymer-drug ratio, surfactant concentration for secondary emulsification process, volume of processing medium and stirring speed of secondary emulsification process was evaluated with respect to entrapment efficiency and *in vitro* drug release behaviors. The *in vitro* release profiles from microspheres of different polymer-drug ratios were applied on various kinetic models. The higher drug loading typically results in lower encapsulation efficiency due to higher concentration gradients resulting the drug to diffuse out of the polymer/solvent droplets to the external processing medium. The viscosity of the polymer solution at higher drug loading was very high and was responsible for the formation of larger polymer/solvent droplets. It caused a decrease rate of entrapment of drug due to slower hardening of the larger particles, allowing time for drug diffusion out of the particles, which tends to decrease encapsulation efficiency. The attempt to prepare controlled release microspheres of zidovudine with increased entrapment efficiency was successful, even though the entrapment efficiency was still lower compared to the same process reported for other hydrophilic drugs.

Amitava Ghosh *et al.,* (**2007**) prepared lamivudine incorporated microspheres composed of ethyl cellulose as release controlling polymeric material. Microspheres were prepared from various methods, namely, modified w/o/o emulsion solvent evaporation method, o/w/o type emulsion solvent evaporation method, thermal change technique, Nobel Quasi emulsion solvent diffusion method, meltable dispersion method, phase separation coacervation method (non-solvent addition technique). The prepared microspheres were evaluated for parameters such as, percentage yield, drug entrapment efficiency, particle size determination, drug polymer interaction, stability studies, and *in vitro* drug release kinetic study. The drug polymer ratio and drug load kept constant throughout the current investigation. Among the methods adopted in this study, thermal change method was most successful in sustaining the release of lamivudine from ethyl cellulose microspheres.

Li Jun *et al.,* (2007) prepared procaine haemoglobin microcapsules of chitosan –sodium alginate. Microcapsules were prepared by using an emulsification –gelation method. Microcapsules possess a relatively narrow and normal Gaussian distribution. The procaine hemoglobin released from microcapsules were extended for more than one month. Chitosan-sodium

alginate –hemoglobin microcapsules were expected to become an artificial oxygen carrying therapeutic agent with SR for intravenous injection.

Tamizharasi *et al.*, (2007) Formulated, characterized and carried out *In-vitro* release kinetics of Aceclofenac loaded poly (ε-caprolactone) microspheres and reported that, drug to carrier ratio-(1:4) showed highest drug entrapment and the drug released up to 15 hour and found to be sustained. There was no interaction between drug and polymer.

Prakash *et al.*, (2007) prepared and evaluated microcapsules for the controlled release of lamivudine using various cellulose polymers. The microcapsules were prepared by the solvent evaporation method. The prepared microcapsules were characterized for the percent drug content, entrapment efficiency, FTIR, DSC, scanning electron microscopy (SEM) and in vitro dissolution studies. The entrapment efficiency was 76-86%. The release of drug from the microcapsules extended up to 8 to 12 hours. FTIR and DSC thermograms showed the stable character of lamivudine in the microcapsules. SEM revealed that the microcapsules were porous in nature. The release kinetics data and characterization studies proved that drug release from microcapsules was diffusion – controlled and that the microcapsules were stable.

Dong Xun Li *et al.,* (2007) prepared ibuprofen microcapsule by spray drying technique. To develop a novel ibuprofen-loaded gelatin microcapsule with bioavailability enhancement, the effect of spray-drying conditions, gelatin, ibuprofen and sodium lauryl sulfate on the ibuprofen solubility and the amount of ethanol encapsulated in gelatin microcapsule were investigated. The ibuprofen solubility and amount of encapsulated ethanol increased as inlet temperature and amount of sodium lauryl sulfate increased, reached maximum at 105°C and 0.6%, respectively and after that followed a rapid decrease. This ibuprofen loaded gelatin microcapsule gave significantly higher initial plasma concentrations, C_{max} and AUC of ibuprofen than did ibuprofen powder, indicating that the drug from gelatin microcapsule could be more orally absorbed in rats. Thus, the ibuprofen-loaded gelatin microcapsule developed using spray drying technique with gelatin was a more effective oral dosage form for poorly water-soluble ibuprofen.

Parul Trivedi *et al.,* (2008) prepared and characterized of aceclofenac microspheres. The microspheres were prepared by o/w emulsion – solvent evaporation technique using Eudragit S 100, RL 100 and RS 100] to provide controlled release and minimizes local side effects by avoiding the drug release in the upper gastro intestinal track. Prepared microspheres were subjected to micromeritic evaluation, drug loading studies, and *in-vitro* drug

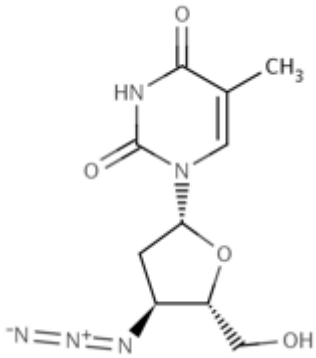
release studies. The drug polymer concentration in the dispersed phase influence the particle size and drug release properties. All the formulations at higher pH follow the Matrix-Higuchi model.

Das *et al.*, (2008) prepared Furosemide loaded alginate microspheres by ionic cross linking technique. Morphology and release characteristics reported that, entrapment efficiency and particle size increased with the increased sodium alginate concentration. The kinetic modeling of the release data indicate that furosemide release from the alginate microspheres follow anomalous transport mechanism after an initial lag period when the drug release mechanism was found to be Fickian diffusion controlled.

DRUG AND POLYMER PROFILE

DRUG PROFILE

ZIDOVUDINE



Empirical formula : $C_{10}H_{13}N_5O_4$

Relative molecular mass : 267.2

Melting point : 106-112°C

Chemical name : 1-[(2R, 4S, 5S)-4-azido-5-(hydroxymethyl) tetrahydrofuran-2-yl]-5-methylpyrimidine-2, 4(1H, 3H)-Dione;

Description	:	A white or brownish powder. A dideoxynucleoside compound in which the 3'-hydroxy group on the sugar moiety has been replaced by an azido group. This modification prevents the formation of phosphodiester linkages which are needed for the completion of nucleic acid chains. The compound is a potent inhibitor of HIV replication, acting as a chain-terminator of viral DNA during reverse transcription.
Solubility	:	Soluble in ethanol (750 g/l) TS (ethanol 95%) , sparingly soluble in water.
Category	:	Anti-HIV Agent, Antimetabolite, Nucleoside and Nucleotide Reverse Transcriptase Inhibitors.(Tripathi., 2008).
Storage	:	Zidovudine should be kept in a tightly closed container, protected from light.

PHARMACODYNAMICS

Mechanism of action

Zidovudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Zidovudine is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated.

Absorption

Rapid and nearly complete absorption from the gastrointestinal tract following oral administration; however, because of first-pass metabolism, systemic bioavailability of zidovudine capsules and solution is approximately 65% (range, 52 to 75%). Bioavailability in neonates up to 14 days of age is approximately 89%, and it decreases to approximately 61% and 65% in neonates over 14 days of age and children 3 months to 12 years, respectively. Administration with a high-fat meal may decrease the rate and extent of absorption.(Tripathi., 2008).

Half life

0.5-3 hours.

Distribution

The apparent volume of distribution of zidovudine, following oral administration, is 1.6 ± 0.6 L/kg; and binding to plasma protein is low, < 38%.

Biotransformation

Hepatic metabolized by glucuronide conjugation to major, inactive metabolite, 3′-azido-3′-deoxy-5′- O-beta-D-glucopyranuronosylthymidine (GZDV).

Elimination

Zidovudine is eliminated from the body primarily by renal excretion following metabolism in the liver (glucuronidation). In patients with severely impaired renal function (CrCl<15 mL/min), dosage reduction is recommended. Although the data are limited, zidovudine concentrations appear to be increased in patients with severely impaired hepatic function which may increase the risk of hematologic toxicity.

Drug interaction

Concomitant use of zidovudine with stavudine should be avoided since an antagonistic relationship has been demonstrated.

Some nucleoside analogues affecting DNA replication, such as ribavirin, antagonize the antiviral activity of zidovudine against HIV-1;

concomitant use of such drugs should be avoided.

Concomitant use of zidovudine with doxorubicin should be avoided since an antagonistic relationship has been demonstrated.

Precautions

Zidovudine is eliminated from the body primarily by renal excretion following metabolism in the liver (glucuronidation).

Patients should be informed that the major toxicities of zidovudine are neutropenia and/or anemia.

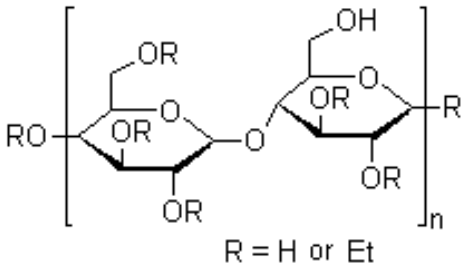
Patients should be informed that other adverse effects of zidovudine include nausea and vomiting.

Adverse effects

- Asthenia
- Headache
- Malaise
- Anorexia
- Constipation
- Nausea
- Vomiting

POLYMER PROFILE

ETHYL CELLULOSE



R = H or Et

Synonyms

Aquacoat ECD, Aqualon E 462, ethocel, surelease.

Chemical name

Cellulose ethyl ether

Description

Ethocel is tasteless, free flowing, white to light tan-coloured powder.

Density

0.4 g/cm^3.

Glass transition temperature

129 -133^o C.

Solubility

Ethyl cellulose is practically insoluble in glycerin, propylene glycol, water.

Ethyl cellulose that contains less than 46.5% of ethoxy group is freely soluble in chloroform, Methyl acetate, tetrahydrofuran, aromatic hydrocarbons and ethanol.

It is also freely soluble in ethanol, ethyl acetate, methanol and toluene.(Handbook of pharmaceutical exicipients, 2003).

Specific gravity

1.12-1.15 g/$cm^{3.}$

Viscosity

5-100 m Pascal (7-100 cp).

Functional category:

Coating agent, flavoring fixative, tablet binder, tablet filler, viscosity increasing agent.

Method of manufacture

Ethyl cellulose is prepared by treating purified cellulose (sourced from chemical-grade cotton, linters and wood pulp) with an alkaline solution, followed by ethylation of the alkali.

The manner ethyl group is added to cellulose can be described by the degree of substitution (DS). The DS designates the average number of hydroxyl positions on the anhydroglucose unit that have been reacted with ethyl chloride. Since each anhydroglucose unit of the cellulose molecule has three hydroxyl groups, the maximum DS is three.

Stability and storage conditions

Ethylcellulose is stable and slightly hygroscopic material. chemically resistant to alkali and more sensitive to acid materials. Ethylcellulose is subjected to oxidative degradation in the sunlight or UV radiation at elevated temperature. This can be prevented by using an antioxidant and chemical additives that absorb light in 230-340 nm range.

It should be stored at a temperature not exceeding 32°C and in the areas away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

Safety

Ethylcellulose is widely used in oral and in topical pharmaceutical formulations. It is also used in food products. Ethylcellulose is not metabolized following oral consumption and therefore it is a non calorific substance. Because ethylcellulose it is not recommended for parenteral formulations; parenteral usage may be harmful to the kidneys.

Ethyl cellulose is generally regarded as a nontoxic non allergenic and nonirritating material. Ethylcellulose intake is not considered as a health hazard and WHO has not specified and acceptable daily intake.

Incompatibilities

It is incompatible with microcrystalline wax and paraffin wax.

Applications in pharmaceutical formulations:

- To mask the unpleasant taste of drug.
- Hydrophobic coating agent for tablets and granules.
- Modified release of the drug.
- To improve the stability of the formulation.
- Used in cosmetics and food products.
- Thickness agent in creams, lotions and gels.
- Binders in tablets.

EXPERIMENTAL WORK

MATERIALS USED

Zidovudine

- Gift sample from Ranbaxy laboratories.

Ethyl cellulose

- Lobachemie

Acetonitrile

- Qualigens

Dichloromethane

- Lobachemie

Light liquid paraffin

- Lobachemie

Span 20

- Lobachemie

n-Hexane

- Lobachemie

INSTRUMENTS AND EQUIPMENTS USED

UV spectrophotometer

- SHIMADZU UV 1650 PC

Electronic balance

- AND HR-200

Dissolution test apparatus

- LAB INDIA, DISSO 2000

FT-IR

- SHIMADZU FTIR-8400 S

Mechanical stirrer

- REMI MOTORS

IR - Hydraulic pellet press

- M - 15 KBr Press

pH Meter

- LI-120, Elico

Scanning electron microscope

- Jeol JSM - 6400

METHODS

Preparation of ethyl cellulose microspheres by double emulsion solvent diffusion technique.

Method Employed

All microspheres were prepared by the w/o/o double emulsion solvent diffusion method .The effect of various formulation and processing factors on microspheres characteristics were investigated by changing polymer-drug ratio and the volume of external oil phase. Weighed amounts of ethylcellulose and zidovudine (AZT) (Different Ethylcellulose: AZT ratios were 1:0.25, 1:0.5, 1:0.75 and 1:1) were dissolved in 5 mL of a mixture of acetonitrile and dichloromethane (1:1). The initial w/o emulsion was formed by adding 2 mL of deionized water to the drug-polymer solution with constant stirring at 500 rpm for 5 min. The w/o primary emulsion was then slowly added to light liquid paraffin (variable volume of 50 mL, 100 mL) containing Span 20 (concentration of 2% w/v) as a surfactant with constant stirring (speed 1000) for 2 h. The n-hexane (10 mL) was added to harden the formed microspheres and the stirring was further continued for 1 h. The resulting microspheres were separated by decantation, freed from liquid paraffin by repeated washing with n-hexane (3 × 50 mL) and finally air dried over a period of 12 h.

Table: 3 Formulation of zidovudine microspheres using Ethylcellulose

S.NO	Formulation	zidovudine (mg)	Ethyl Cellulose (mg)	Drug:polymer Ratio	Light liquid paraffin (ml)
1	F1	250 mg	1000 mg	0.25:1	50
2	F2	500 mg	1000 mg	0.5:1	50
3	F3	750 mg	1000 mg	0.75:1	50
4	F4	1000 mg	1000 mg	1:1	50
5	F5	250 mg	1000 mg	0.25:1	100
6	F6	500 mg	1000 mg	0.5:1	100
7	F7	750 mg	1000 mg	0.75:1	100
8	F8	1000 mg	1000 mg	1:1	100

- **Medium A-** 50 ml light liquid paraffin.
- **Medium B-** 100 ml light liquid paraffin.

PREFORMULATION STUDIES

Standard graph of zidovudine

- Preparation of stock solution:
- 100 mg of zidovudine dissolved in 100 ml of PH 7.4 phosphate buffer to get a concentration of 100 µg/ml.
- From this take 2 ml and made up to 100 ml with PH 7.4 phosphate buffer to get a concentration of solution 20 µg/ml.

Preparation of various concentrations of zidovudine solution

- 1ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 2µg/ml.
- 2ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 4µg/ml.
- 3ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 6µg/ml.
- 4ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 8µg/ml.
- 5ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 10µg/ml.
- 6ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 12µg/ml.
- 7ml of the stock solution was taken was made up to 10 ml with pH

7.4 phosphate buffer to give 14µg/ml.

- 8ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 16µg/ml.
- 9ml of the stock solution was taken was made up to 10 ml with pH 7.4 phosphate buffer to give 18µg/ml.
- 10ml of the stock solution was taken which contains 2µg/ml.

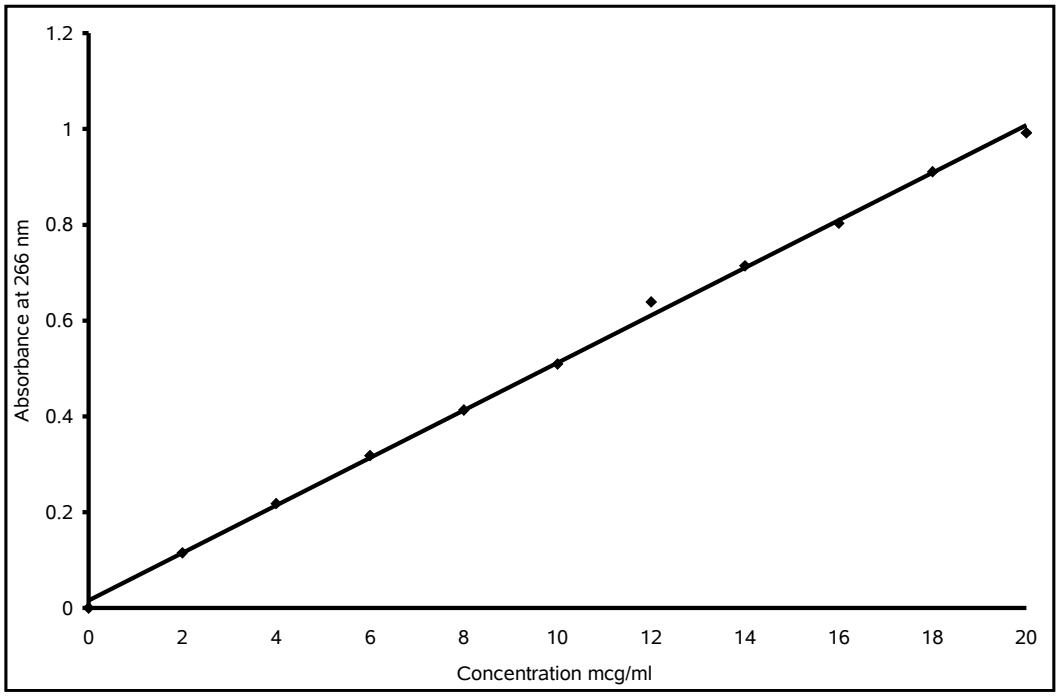
Procedure

Various concentrations of zidovudine (2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/ml) were prepared as mentioned above. Absorbance of the solution was measured against reagent blank at 266 nm using UV spectrophotometer. A standard graph between concentration Vs absorbance was plotted. A straight line passing through the origin was obtained.

Table 4 : Calibration data for the estimation of zidovudine (2-20 µg/ml)

Concentration (µg/ml)	Absorbance at 266 nm.
0	0.0000
2	0.1151
4	0.2180
6	0.3181
8	0.4132
10	0.5092
12	0.6391
14	0.7140
16	0.8030
18	0.9107
20	0.9920

Fig. 3 Stadard graph of Zidovudine



INTERACTION STUDY

Equipment

1. IR- hydraulic pellet press
2. Perkin Elmer FTIR.

Infrared (IR) absorption spectroscopy

To investigate any possible interactions between the drug and the polymers, the IR spectra of pure drug zidovudine and its physical mixtures (1:1) with Ethylcellulose were carried out using shimadzu IR-470 spectrophotometer (Tokyo, Japan).The samples were prepared as KBr disks compressed under a pressure of 6 ton/nm^2. The wavelength selected ranged between 400-4000 cm^{-1} in a Perkin Elmer FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with those of pure drug and polymers and matching was done to detect any appearance or disappearance of peaks.

Procedure

First a pinch of drug was added to the dried KBr, and triturated in mortar. The pellet was made by using pellet plate technique, and kept in IR chamber and scanned to get the spectra. The same procedure was followed for the combination of drug-polymer too.

By comparing the IR spectrum of drug and drug-polymer combinations, it was inferred that in both cases the characteristic peaks were obtained at same wave numbers. So from this it was concluded that no structural changes had occurred for the drug when combined with the polymers.

Fig. 4 IR Specrum of Zidovudine

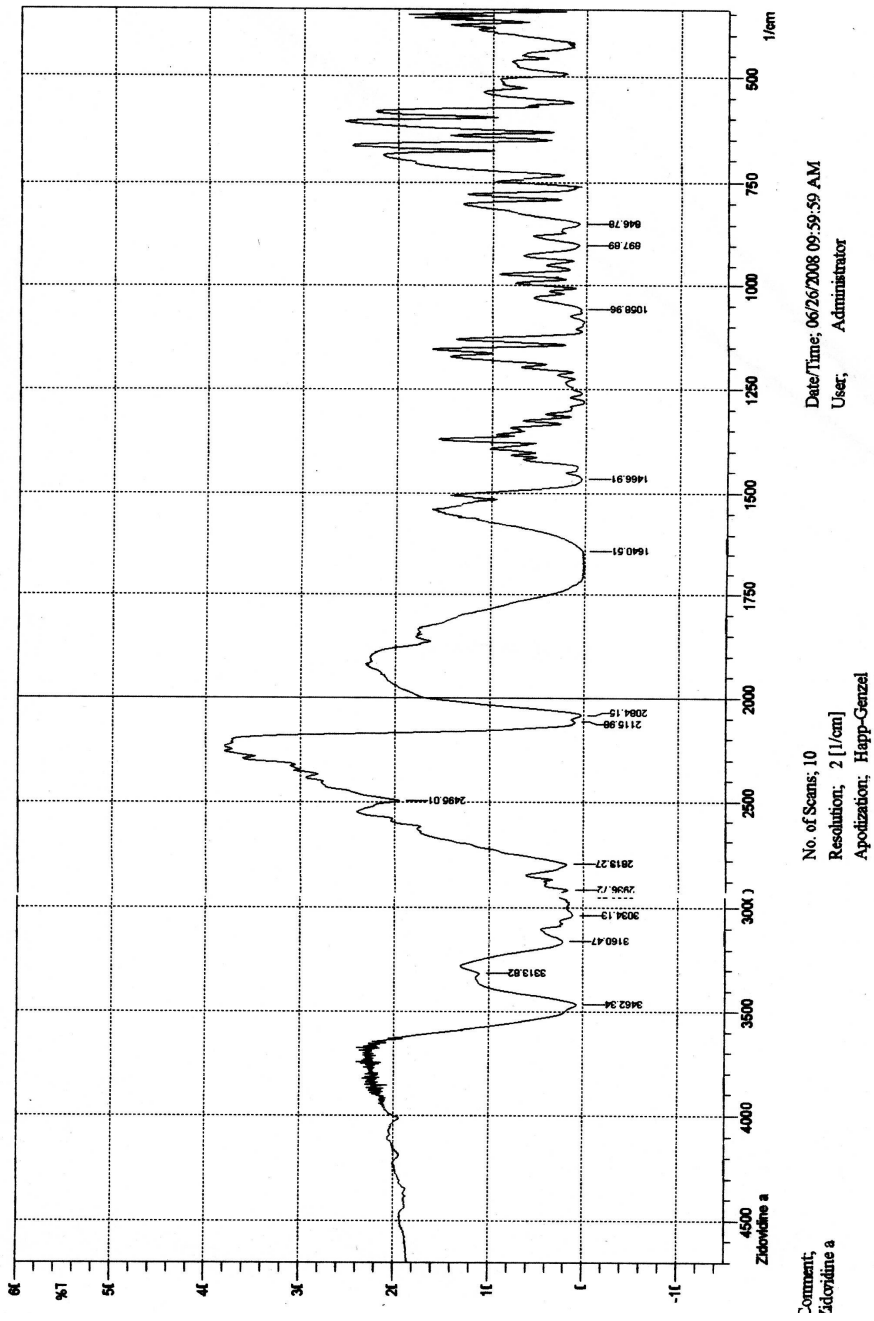


Fig. 5 IR Specrum of Ethylcellulose

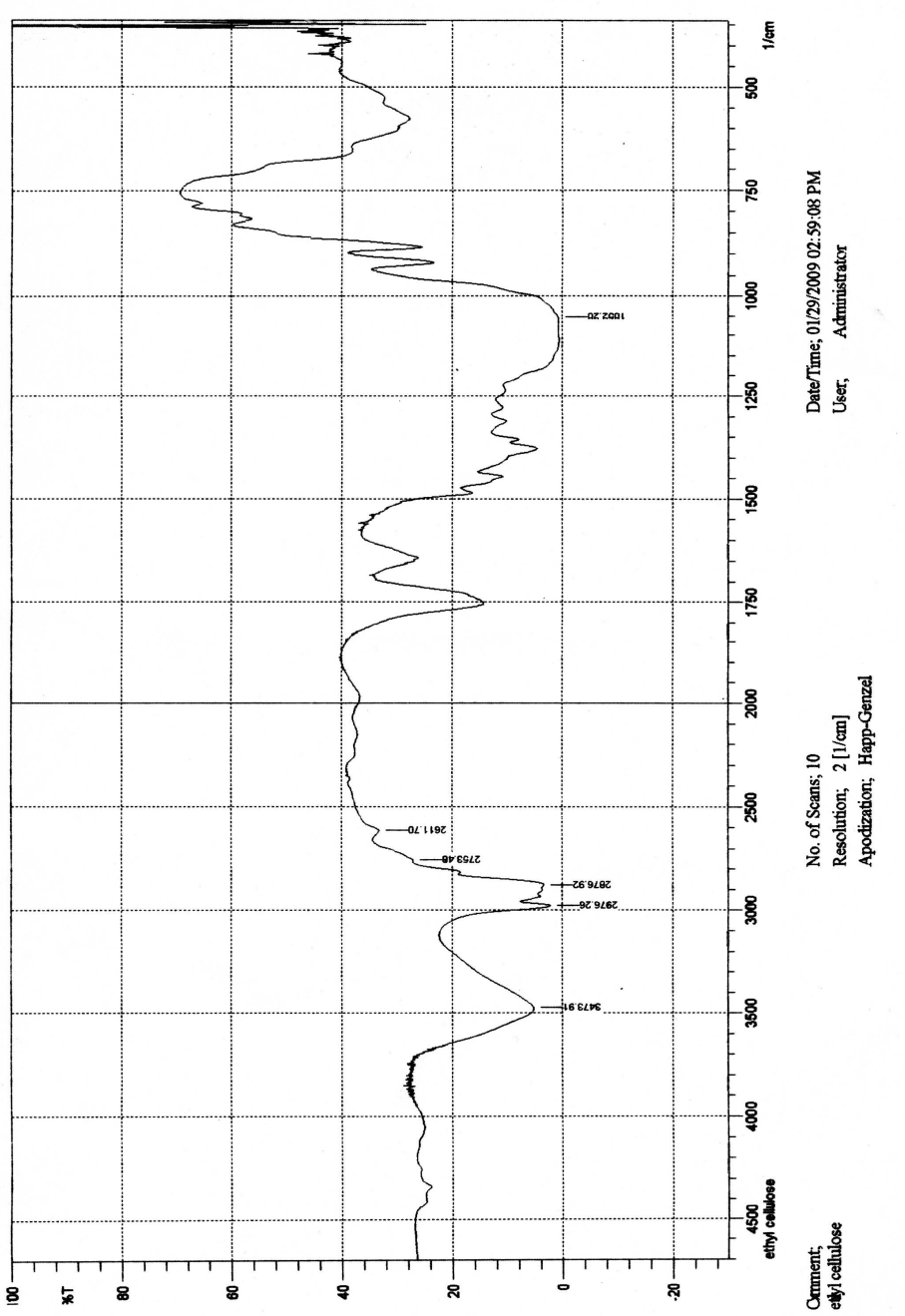
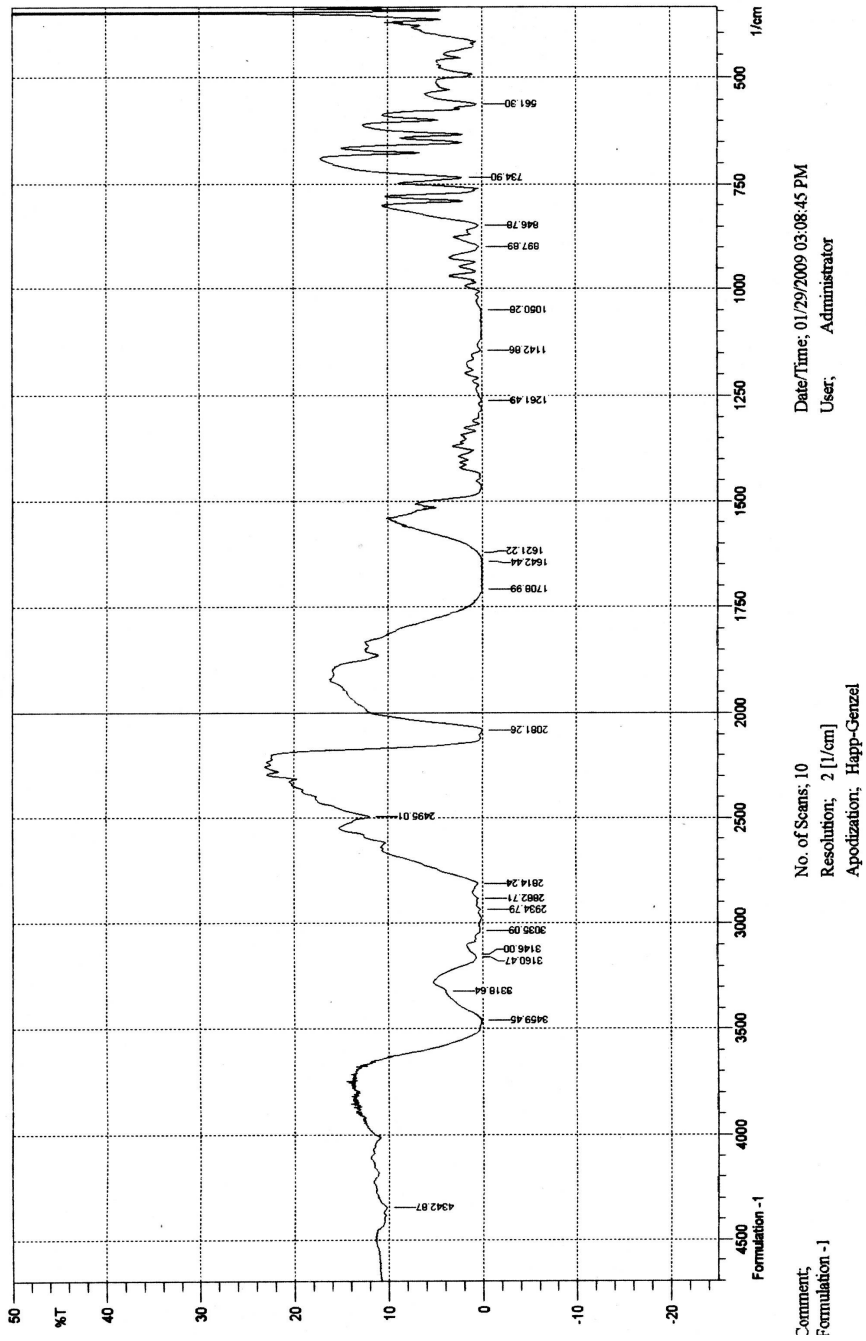


Fig. 6 IIR SPECTRUM OF FORMULATION



Results and Discussion

The compatibility between zidovudine and the selected polymer ethyl cellulose was evaluated using FTIR peak matching method. The IR spectra of pure drug, polymer and physical mixtures are shown in spectra above (fig.4, fig.5 and fig.6) respectively. There was no disappearance of peak in polymer drug mixture, which confirmed the absence of any chemical interaction between drug and polymer.

Drug content analysis

UV spectrophotometric method was employed to verify the presence of drug in microspheres.30 mg of formulation was taken and the drug was extracted with phosphate buffer pH 7.4 and absorbance was measured using UV spectrophotometer at 266nm. The amount of zidovudine in the microspheres was estimated with the help of standard graph. A study was performed on the percentage yield and percentage encapsulation efficiency.

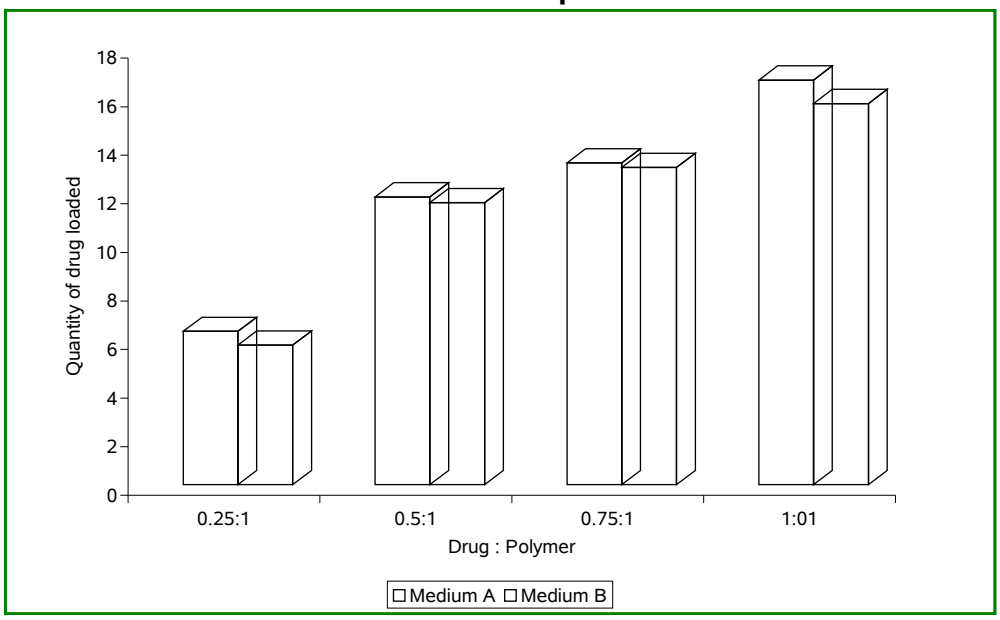
Drug encapsulation efficiency =

$$\frac{Amount\ \ of\ \ drug\ \ bound\ \ to\ \ the\ \ microspheres\,(mg)}{Total\ \ amount\ \ of\ \ applied\ \ drug\,(mg)}$$

Table 5 : Amount of drug loaded in Ethylcellulose microspheres using 50 and 100 ml of dispersion medium.

Drug:Polymer	Amount of drug loaded	
	50 ml of dispersion medium	100 ml of dispersion medium
0.25:1	6.31±0.5	5.74±0.5
0.5:1	11.85±0.3	11.61±0.7
0.75:1	13.25±0.5	13.06±0.6
1:1	16.66±0.5	15.69±0.2

Fig. 7 Amount of drug loaded in Ethylcellulose microspheres using 50 and 100 ml of dispersion medium



Results and Discussion

The amount of drug loaded in the microspheres prepared using 50 and 100 ml of the light liquid paraffin was represented in fig.7. It was found that the amount of drug getting loaded into the microspheres increased when the amount of drug used for the preparation of the microspheres. The amount of drug getting loaded was found to be 6.31±0.5, 11.85±0.3, 13.25±0.5 and 16.66±0.5 mg when the volume of dispersion medium was low i.e. 50ml and 5.74±0.5, 11.61±0.7, 13.06±0.6 and 15.69±0.2 mg when the volume of dispersion medium was high i.e. 100ml.

From the above results it was found that the amount of drug getting loaded into the microspheres was high when the volume of medium was low and less when the volume of medium was high.

The polymer ratio was found to be inversely proportional to drug loading capacity.

Percentage Encapsulation efficiency of ethylcellulose microspheres

The amount of zidovudine present in the microspheres was determined by extracting into phosphate buffer (pH 7.4). 40 mg of microspheres were crushed and powdered by using a mortar and pestle and 30mg of accurately weighed powder was extracted into 100ml of phosphate buffer (pH 7.4) by stirring at 1000 rpm for 2 h. The solution was filtered, diluted 100 times and estimated for zidovudine content using UV/Visible spectrophotometer at 266 nm. (Das et al., 2005).

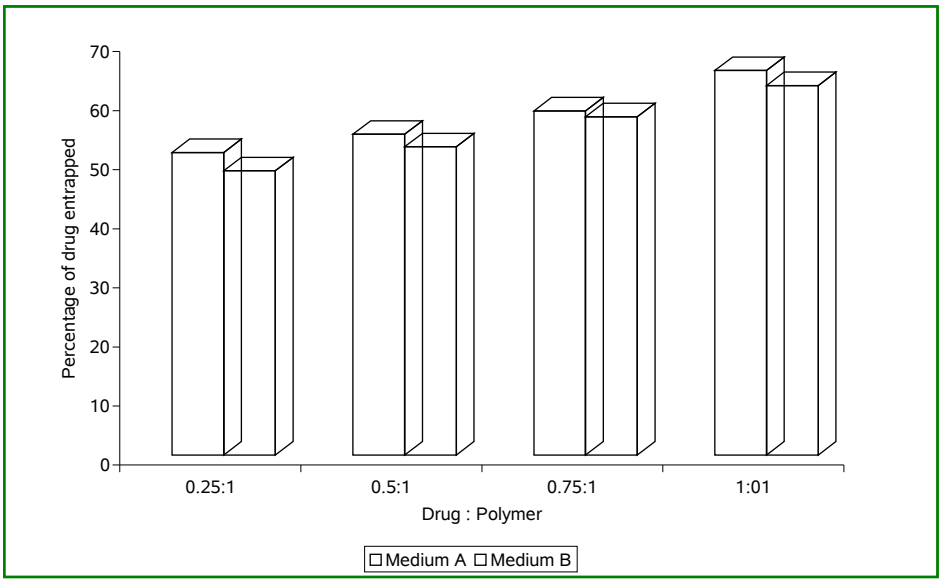
Percentage entrapped

$$\textbf{(\%) =} \quad \frac{Amount\ \ of\ \ drug\ \ bound\ \ to\ \ the\ \ microspheres\,(mg)}{Total\ \ amount\ of\ \ applied\ \ drug\,(mg)} \times 100$$

Table 6: Percentage encapsulation efficiency of ethyl cellulose microspheres using 50 and 100 ml of dispersion medium

Drug:Polymer	Percentage of drug loaded	
	50 ml of dispersion medium	100 ml of dispersion medium
0.25:1	51.22±0.5	48.12±0.6
0.5:1	54.29±0.6	52.18±0.2
0.75:1	58.26±0.3	57.27±0.5
1:1	65.12±0.5	62.57±0.4

Fig. 8 Percentage encapsulation efficiency of ethyl cellulose microspheres using 50 and 100 ml of dispersion medium



Results and Discussion

The percentage of drug loaded in the microspheres prepared using 50 and 100 ml of the light liquid paraffin was represented in fig.8. It was found that the percentage of drug getting loaded into the microspheres was directly proportional to the amount of drug. The amount of drug getting loaded was found to be 51.22±0.5, 54.29±0.6, 58.26±0.3 and 65.12±0.8 % when the volume of dispersion medium was low i.e. 50ml and 48.12±0.6, 52.18±0.2, 57.27±0.5 and 62.57±0.4 % when the volume of dispersion medium was high i.e. 100ml.

From the above results it was found that the percentage of drug getting loaded into the microspheres was high when the volume of medium was low and less when the volume of medium was high.

Percentage yield of ethylcellulose microspheres

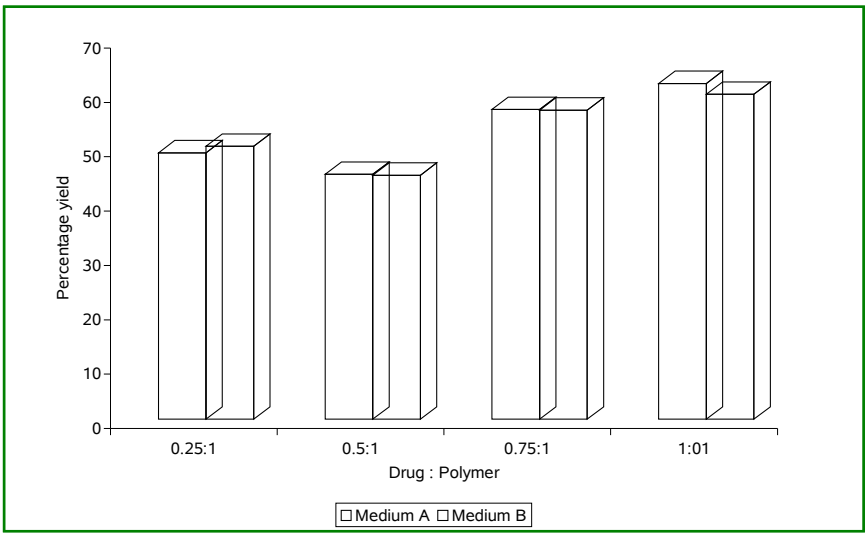
The percentage yield of the microspheres was calculated by accurately weighing out the amount of product obtained at the end of each batch of each batch of formulation. The formula given below was used to calculate the % yield of the microspheres. (Sunit Kumar Sahoo et al., 2005).

Percentage yield (%) = $$\frac{Amount\ \ of\ \ recored\ \ microparticle\,(mg)}{(The\ \ theoratical\ \ content)\,(mg)} \times 100$$

Table 7: Percentage yield of ethylcellulose microspheres using 50 and 100 ml of dispersion medium

Drug:Polymer	Percentage yield	
	50 ml of dispersion medium	100 ml of dispersion medium
0.25:1	49.04	50.24
0.5:1	45.08	44.9
75:1	57.0	56.9
1:1	61.8	59.8

Fig. 9 Percentage yield of Ethylcellulose Microspheres using 50 and 100 ml of dispersion medium.



Results and Discussion

The percentage yield of microspheres by using various drug polymer ratio and different volume of dispersion medium was shown in fig 9. The percentage yield in 50 ml of dispersion medium was found to be 49.04%, 45.08%, 57.0% and 61.8%. The percentage yield in 100 ml of distribution medium was found to be 50.24%, 44.9%, 56.9% and 59.8%.

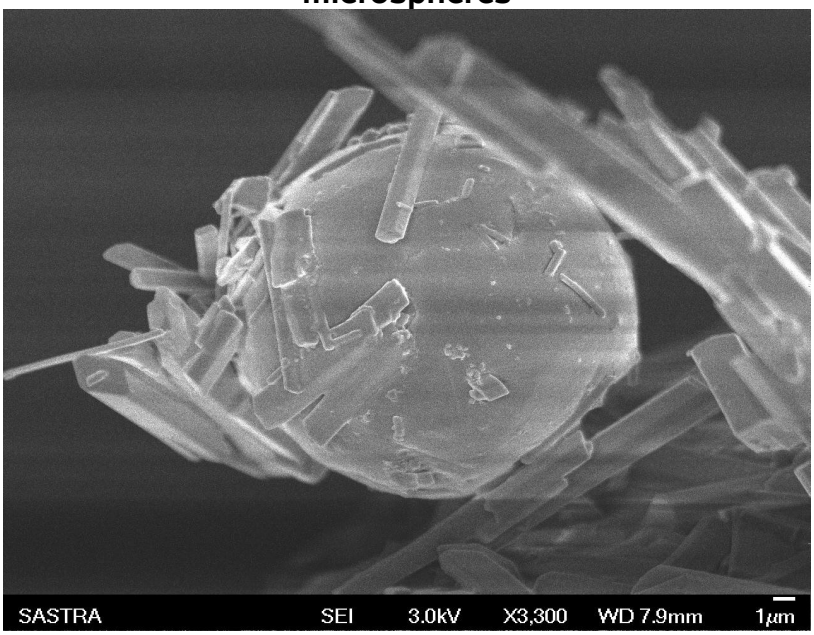
From the above results it was found that the percentage yield was high when the volume of dispersion medium used was 50 ml.

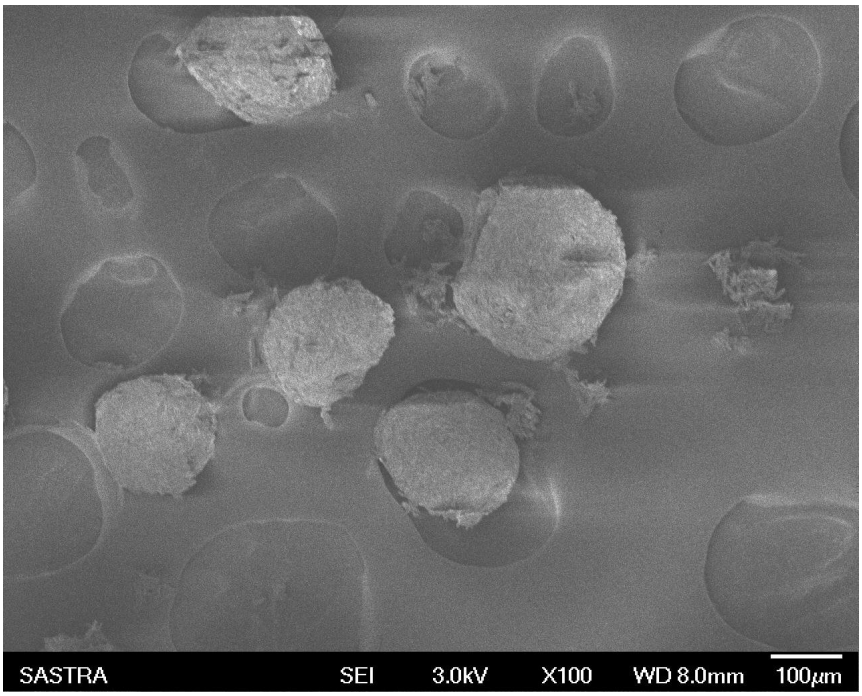
DETERMINATION OF SIZE AND SHAPE OF MICROSPHERES

Determination of size and shape of microspheres using scanning electron microscope:-

The surface morphology and internal texture of zidovudine microspheres were observed by a scanning electron microscope (SEM). SEM photographs were taken on 3kv magnification at 18°C. Before scanning the particles were sputtered with platinum to make the surface conductive. The photograph shows that the particles are spherical enough and have a smooth surface.

Fig. 10, 11 Scanning electron microscope pictures of ethyl cellulose microspheres





Measurement of particle size determination of microspheres by optical microscopy method:-

Microscope was cleaned and focused for bright light eyepiece was fixed in microscope with micrometer. Eyepiece micrometer was calibrated using standard stage micrometer. Few drops of sample were mounted in a glass slide. Particle size was measured using standard stage micrometer. 100 particles were tabulated for predicting the average mean diameter and particle size distribution.

Arithmetic mean = **Σnd/Σn**

Table: 8 calibration of eyepiece micrometer

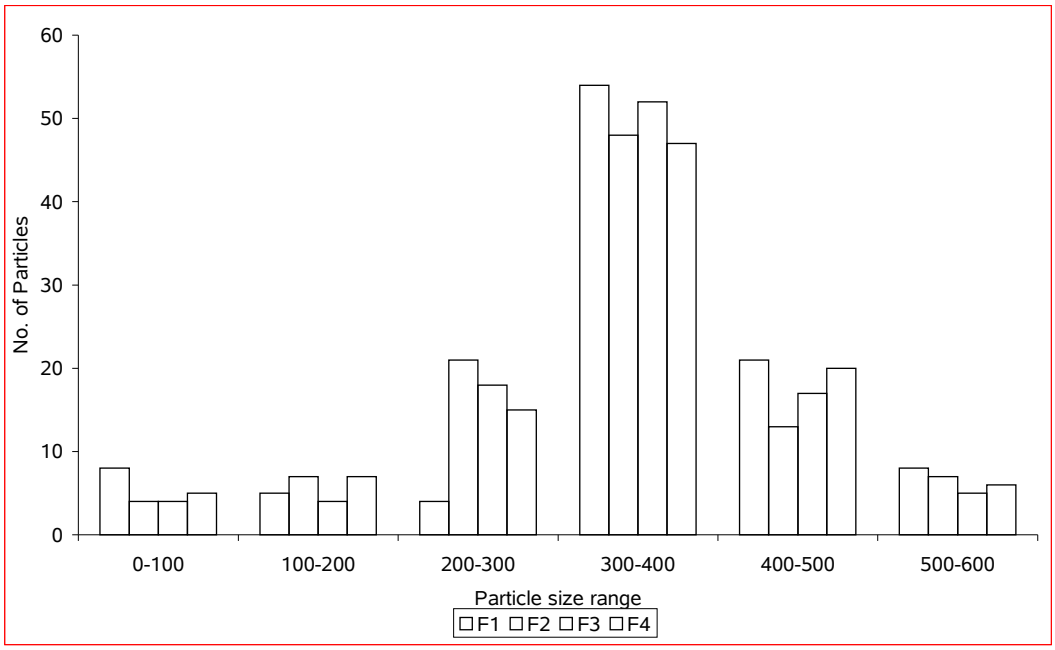
Stage Micrometer	0	13.4	18.8	26.8	47
Eyepiece Micrometer	0	10	14	20	35

Table: 9 Particle size determination by optical microscopic method for formulations prepared using 50 ml dispersion medium

Formulation code	Particle size range (μm)	Mean diameter (μm) (d)	Number of particles (n)	nxd
F1	0-100	50	8	400
	100-200	150	5	750
	200-300	250	4	1000
	300-400	350	54	18900
	400-500	450	21	9450
	500-600	550	8	4400
F2	0-100	50	4	200
	100-200	150	7	1050
	200-300	250	21	5250
	300-400	350	48	16800
	400-500	450	13	5850
	500-600	550	7	3850
F3	0-100	50	4	200
	100-200	150	4	600
	200-300	250	18	4000
	300-400	350	52	18200
	400-500	450	17	7650
	500-600	550	5	2750
F4	0-100	50	5	250
	100-200	150	7	1050
	200-300	250	15	3750
	300-400	350	47	16450
	400-500	450	20	9000
	500-600	550	6	3300

Fig.12 Particle size determination by optical microscope method using

for formulations prepared using 50 ml dispersion medium

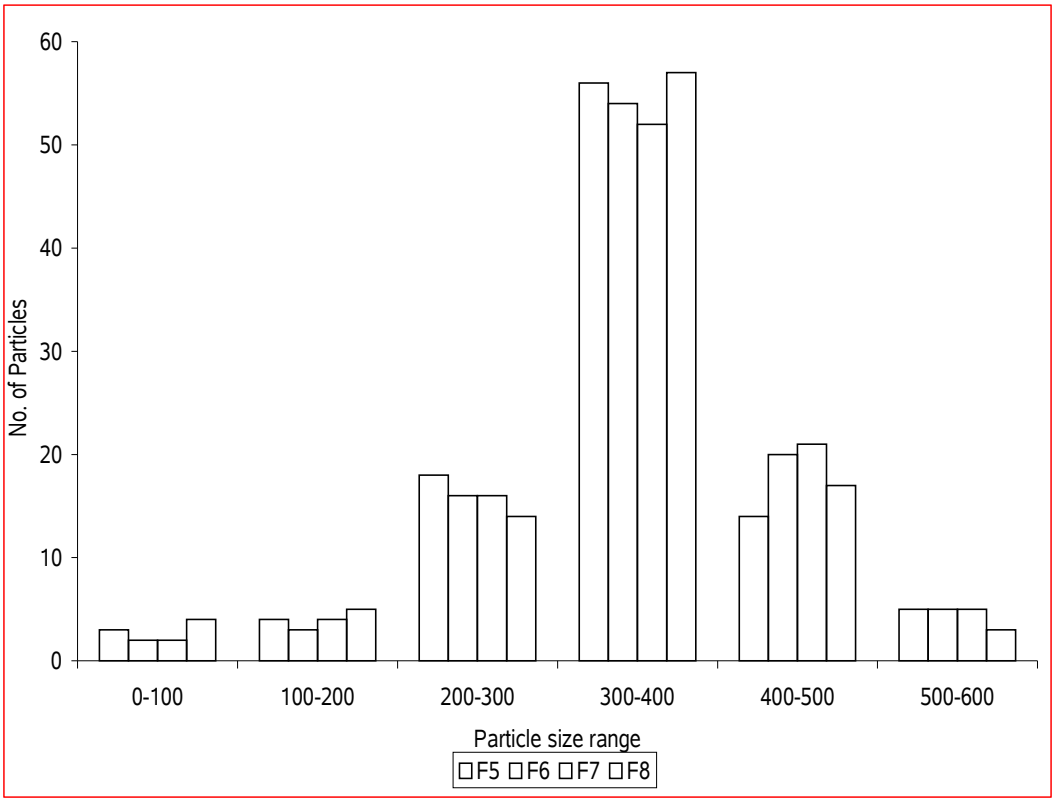


Results and Discussion

The number of particles present in each size range was shown in fig 12 for the formulations made using 50 ml of light liquid paraffin and drug polymer ratio of (0.25:1, 0.5:1, 0.75:1, 1:1). The highest number of particles were found in the range of 300-400 µm.

Table: 10 Particle size determination by optical microscopic method for formulations prepared using 100 ml dispersion medium.

Fig. 13 Particle size determination by optical microscope method for formulations prepared using 100 ml dispersion medium



Results and discussion

The number of particles present in each size range was shown in fig 13 for the formulations made using 100 ml of light liquid paraffin and drug polymer ratio of 0.25:1, 0.5:1, 0.75:1 and 1:1. The highest number of particles was found in the range of 300-400 µm.

Arithmetic mean:

F1 = 349.00 µm	**F 5= 307.00 µm**
F 2= 330.00 µm	**F 6= 352.00 µm**
F 3= 334.00 µm	**F 7= 351.00 µm**
F 4= 338.00 µm	**F 8= 337.00 µm**

4.5 *In Vitro* Drug Release Studies

The USP basket-type dissolution (Type II) test apparatus was used for *in vitro* release studies. A weighed quantity of the microspheres was suspended in 900 ml of phosphate buffer (pH 7.4). The dissolution medium was stirred at 100 rpm and maintained at constant temperature (37 ± 1^{0} C). At preset time intervals 5 ml aliquots were withdrawn at 0, 1, 2, 4, 6, 12, and 24 hours and replaced by an equal volume of fresh dissolution medium to maintain sink condition. After suitable dilution, the samples were analyzed at 266 nm using UV-Visible spectrophotometer.

Calculation

To calculate the actual percentage release of drug it is necessary to calculate the theoretical release of the drug. It can be calculated by using the general formula,

Theoretical release = (total amount o drug loaded in 1mg of microspheres) x (amount of microspheres taken for in vitro studies)

The exact percentage release of drug can be obtained by the general formula:

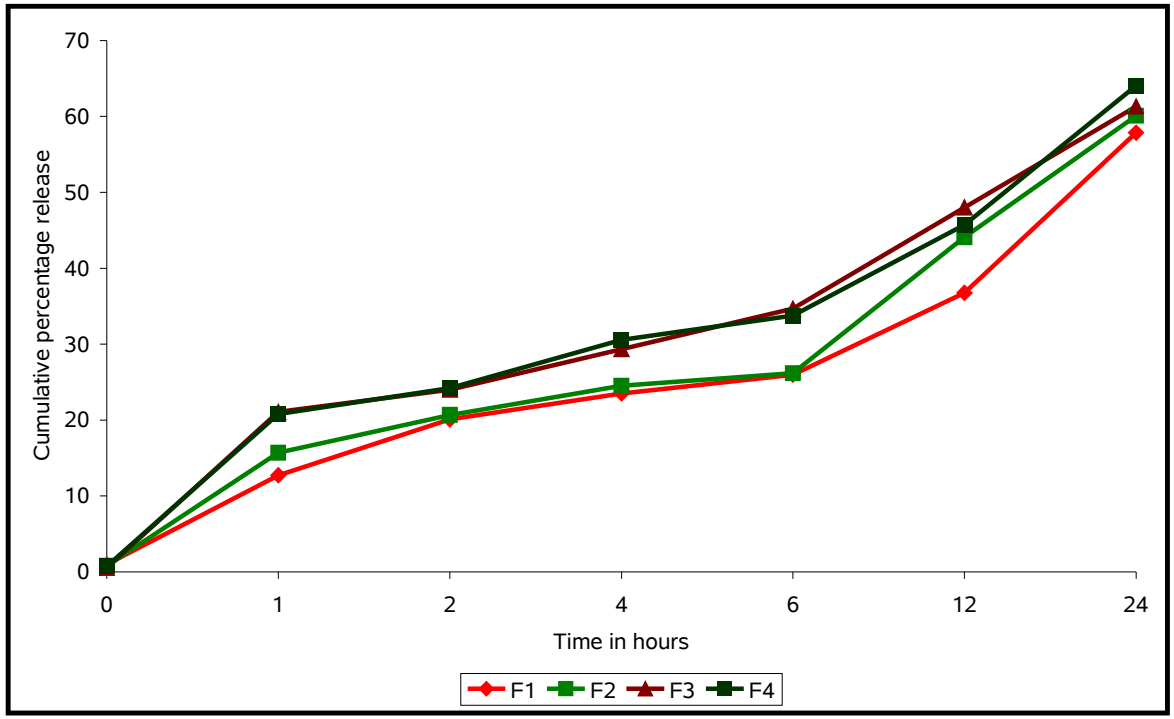
Exact percentage release or
Cumulative release $= \frac{Actual\ \mathrm{Re}lease}{Theoretical\ \mathrm{Re}lease}\ X\ 100$

IN VITRO DRUG RELEASE STUDIES

Table: 11 Percentage of drug released for formulations prepared using 50 ml of dispersion medium.

S.No	Time(hrs)	Cumulative percentage release			
		F1	F2	F3	F4
1	0	0.98±0.02	0.82±0.08	0.53±0.05	0.73±0.04
2	1	12.74±0.04	15.7±0.05	21.06±0.07	20.78±0.05
3	2	20.09±0.06	20.66±0.07	24.0±0.05	24.2±0.08
4	4	23.52±0.05	24.51±0.04	29.33±0.04	30.56±0.05
5	6	25.98±0.07	26.17±0.05	34.66±0.08	33.74±0.04
6	12	36.76±0.07	44.07±0.07	48.0±0.04	45.72±0.07
7	24	57.85±0.08	60.06±0.04	61.3±0.05	64.05±0.08

Fig. 14 Percentage release of zidovudine microspheres prepared using 50 ml of dispersion medium



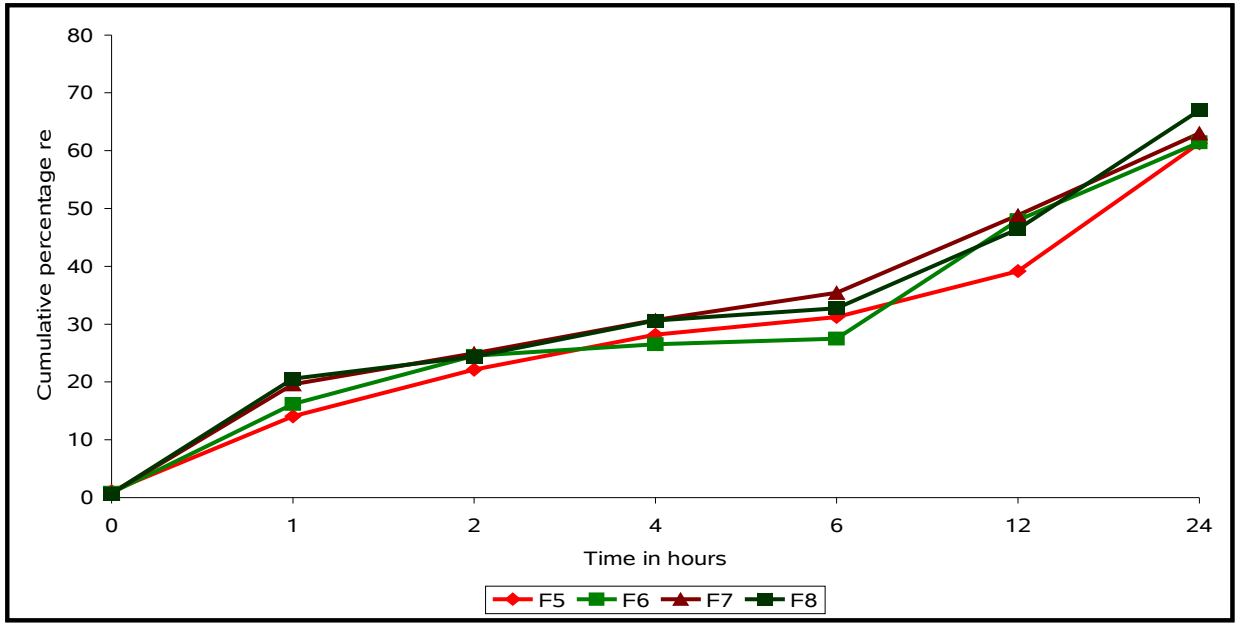
Results and Discussion:

The *in vitro* release profile was shown in fig.14. All the formulation was found to show sustained release. The release profile of F1, F2, F3 & F4 was found to be 57.85%, 60.06%, 61.3% & 64.05% respectively at the end of 24 hours. The results suggest that the polymer concentration having a significant influence on the release profile. It was found to be inversely proportional. As the drug polymer ratio increase there was a low release profile. 1:1 drug polymer ratio can be further evaluated *in vivo* to optimize the formulation.

Table : 12 Percentage of drug released for formulations prepared using 100 ml of dispersion medium.

S.No	Time (hrs)	Cumulative percentage release			
		F5	F6	F7	F8
1	0	1.1±0.03	0.8±0.06	0.78±0.05	0.71±0.03
2	1	14.07± 0.04	16.17± 0.18	19.56± 0.06	20.57± 0.08
3	2	22.11±0.08	24.52±0.08	24.93±0.06	24.4±0.05
4	4	28.14±0.06	26.51±0.03	30.7±0.05	30.62±0.06
5	6	31.25±0.05	27.49±0.06	35.43±0.08	32.73±0.03
6	12	39.19±0.07	47.97±0.03	48.81±0.05	46.41±0.08
7	24	61.3±0.08	61.45±0.05	62.99±0.07	66.94±0.03

Fig. 15 Percentage release of zidovudine microspheres prepared using 100 ml of dispersion medium



Results and Discussion

The *in vitro* release profile was shown in Fig.15. All the formulation was found to show sustained release. The release profile of F5, F6, F7 & F8 was found to be 61.3%, 61.45%, 62.99% & 66.94% respectively at the end of 24 hours. The results suggest that the polymer concentration having a significant influence on the release profile. It was found to be inversely proportional. As the drug polymer ratio increase there was a low release profile. 1:1 drug polymer ratio can be further evaluated *in vivo* to optimize the formulation.

SUMMARY AND CONCLUSION

SUMMARY

Novel drug delivery system is finding more attention in pharmaceutical industries due to its various advantages. In the line sustained/controlled release formulations are more focused for improving the drug bioavailability with less dosing frequency. Zidovudine is one such compound having a poor bioavailability with high dosing frequency. The present investigation is focused to develop a suitable sustained release formulation for achieving better bioavailability and reduce dosing frequency.

Compatibility Studies

Zidovudine was formulated as microspheres using ethylcellulose. Compatibility studies were carried out using FTIR. The results suggest no evidence of any interaction. Different batches containing various concentrations of drug, polymer with varying volume of dispersion medium was prepared by double emulsion solvent diffusion technique.

In Vitro release studies

The *in vitro* release suggest that low volume of dispersion medium and low polymer ratio (F4) showed 64.05% of release at the end of 24 hrs with 65.12 % entrapment.

CONCLUSION

It is evident that zidovudine loaded ethylcellulose microspheres showed better sustained release profile which is up to 24 hrs. The prepared formulation can be further evaluated to achieve a better bioavailability. *IN VIVO* studies can be carried out to optimize the Zidovudine microspheres using ethyl cellulose. The pharmacokinetic behavior of the formulated microspheres can be studies for optimization of the formulation.

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